## **ENVIRONMENT, HEALTH, AND BEHAVIOR**

# Effects of High Zinc Diets Using Zinc Propionate on Molt Induction, Organs, and Postmolt Egg Production and Quality in Laying Hens

S. Y. Park,\*,1 S. G. Birkhold,\* L. F. Kubena,† D. J. Nisbet,† and S. C. Ricke\*

\*Department of Poultry Science, Texas A & M University, College Station, Texas 77843; and †USDA-ARS, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit, College Station, Texas 77845

**ABSTRACT** This study was conducted to determine the ability of an alternative salt form of 1% Zn, Zn propionate, to induce molt in 66-wk-old hens. The hens were randomly assigned to 4 treatment groups of 27 or 28 birds each: a) molted conventionally by feed withdrawal, b) 1% Zn as Zn acetate, c) 1% Zn as Zn propionate, or d) nonmolted control for 9 d. Feed intake was (P < 0.05) depressed in Zn acetate and Zn propionate hens when compared with nonmolted control hens during the 9 d. Ovary weights of hens undergoing feed withdrawal, Zn acetate, or Zn propionate were not (P > 0.05) different from each other, but all were (P < 0.05) lighter than the ovary weights of nonmolted control hens. Zinc concentra-

tions in the kidney and liver were (P < 0.05) increased in Zn acetate and Zn propionate molted hens when compared with nonmolted hens on the control diet or hens molted by feed withdrawal. Bone ash values were (P < 0.05) increased for Zn acetate and Zn propionate molted hens or nonmolted control hens as compared with molted hens on feed withdrawal. Over the entire 3-mo postmolt period, there were no significant differences in interior egg qualities, but egg weights from hens fed Zn propionate were (P < 0.05) heavier than those from hens on feed withdrawal. The data of the current study demonstrated that feeding a Zn propionate (1% zinc)-supplemented diet can induce molt.

(Key words: egg production, egg quality, induced molt, organ, zinc propionate)

2004 Poultry Science 83:24-33

#### INTRODUCTION

In nature, all adult avian species undergo annual bird molting to renew their feathers. This results in BW losses up to 40% of their mass (Mrosovsky and Sherry, 1980) and a pause in oviposition due to regression of the reproductive tract (Mrosovsky and Sherry, 1980).

In the US commercial layer industry, older hens can be artificially induced to molt before the end of a first laying cycle, rest, and enter into a second egg-laying cycle (North and Bell, 1999). After completion of the process of induced molting, old birds exhibit rejuvenation of the reproductive tracts (Brake, 1993). The most commonly practiced method of molt induction is withdrawal of feed for several days. This method efficiently induces a molt because it is management-friendly and economically advantageous and results in satisfactory postmolt performance for the commercial layer industry (Brake, 1993). However, increased public awareness of the animal stress associated with feed withdrawal has led researchers to investigate alternative molting processes. Additionally,

the stress results in increased susceptibility to *Salmonella enteritidis* infection (Holt and Porter, 1992; Holt, 1993; Holt et al., 1995), which may lead to increased risk of foodborne illness to consumers of these products.

Molt induction by feeding hens a diet containing high levels, 10,000 to 20,000 ppm, of added Zn as Zn oxide or Zn acetate results in egg production cessation within 5 d (Scott and Creger, 1976; Creger and Scott, 1977). Berry and Brake (1985) reported that hens fed diets high in Zn stopped ovulating up to a full day sooner than did fasted hens. In several studies it has also been reported that the effectiveness of Zn to induce follicular atresia and halt egg laying is probably caused by this cation's ability to depress feed intake (Scott and Creger, 1976; Shippee et al., 1979; Berry and Brake 1985; McCormick and Cunningham, 1987).

Propionic acid has been used as a feed fungistat, but high concentrations may reduce chicks' feed intake by decreasing palatability (Ryś and Koreleski, 1974; Paster, 1979; Cave, 1982, 1984). Previously reported methods of dietary Zn for induction molt were restricted to Zn acetate and Zn oxide. Therefore, the objective of this study was to investigate whether Zn propionate, an alternative salt

<sup>©2004</sup> Poultry Science Association, Inc.
Received for publication May 30, 2003.
Accepted for publication September 30, 2003.

¹To whom correspondence should be addressed: sricke@poultry.tamu.edu.

 $<sup>\</sup>label{eq:Abbreviation Key: ULACC = University Lab Animal Care Committee.} Abbreviation Key: ULACC = University Lab Animal Care Committee.$ 

form of Zn, would induce molt and affect organ systems, egg quality, and egg production as the hens entered the second cycle of laying in production.

#### MATERIALS AND METHODS

#### General

One hundred forty-two Single Comb White Leghorn (SCWL) hens, 66 wk of age, were obtained from a commercial laying facility. Hens were housed 1 per cage (30  $\times$  35 cm). The hens were maintained under an artificial lighting program of 16L:8D. They were provided access ad libitum to a complete layer ration and water via nipple drinkers to prevent cross-contamination of water and feed for 8 wk prior to molting. The individual feeders were stainless steel. During the 8-wk acclimation period, egg production was monitored to insure that all hens were healthy and in active production.

## **Molting Procedure**

After acclimation, 110 hens were moved into a nearby house and housed 2 per cage for the molting procedure. Hens were assigned to 1 of the following 4 treatment groups: 27 hens to a Zn acetate diet (1% Zn added to layer ration), 27 hens to a Zn propionate diet (1% Zn added to layer ration), 28 hens fed a layer ration without additives to serve as the nonmolted control, or 28 hens to feed withdrawal (negative control). All hens were allowed access to water ad libitum and their respective diets. Hens were placed on an artificial lighting program of 8L:16D for 1 wk prior to molting procedure. Treatments were randomly assigned to cages throughout the house to ensure there was no variability in egg production or reproductive tract regression due to light stimulation.

During the molt procedure, hen weights were monitored at d 1, 3, 5, 7, and 9. In accordance with Texas A&M University Lab Animal Care Committee (ULACC) animal use protocols, any hen reaching 25% weight loss prior to the end of the trial (d 9) was removed from her respective diet and immediately placed on full feed.

After molting, the remaining hens were placed on fed layer ration (Table 1) ad libitum, and the light program was changed to an artificial program of 16L:18D to stimulate egg production. Daily egg production was monitored for 12 wk after the molting diets were replaced with full feed.

## Collection of Organs and Determination of Crop pH

At the end of the molt procedure, 55 hens were euthanized with CO<sub>2</sub> gas according to the approved ULACC

TABLE 1. Composition of Texas A&M University (TAMU) layer ration

| Ingredient                        | Amount (g/kg of mash) <sup>1</sup> |
|-----------------------------------|------------------------------------|
|                                   | (8, 118 01 11111011)               |
| Corn, yellow                      | 567.18                             |
| Soybean meal                      | 316.33                             |
| Vegetable oil                     | 76.82                              |
| Mono calcium phosphate            | 16.86                              |
| Calcium carbonate                 | 15.62                              |
| Methionine, 98%                   | 1.69                               |
| Vitamine premix <sup>2</sup>      | 2.50                               |
| NaCl                              | 2.50                               |
| Trace mineral premix <sup>c</sup> | 0.5                                |
| Total                             | 1,000.00                           |

<sup>&</sup>lt;sup>a</sup>For diet formulations, crude fat concentrations were fixed at 100 g/sg.

 $^2\mathrm{Provides}$  mg/kg of diet unless otherwise noted: vitamin A, 8,818 IU; vitamin D, 2,205 IU; vitamin E, 5.86 IU; vitamin K, 2.2; thiamine, 1.1; riboflavin, 4.4; niacin, 22; pantothenic acid; choline, 500; vitamin B<sub>12</sub>, 0.013; biotin, 0.055.

<sup>c</sup>Trace mineral premix (Nutrius Premix Division, Bioproducts Inc., Cleveland, OH), provided as milligrams per kilogram of diet unless otherwise noted: Mn, 68.2; Zn, 55; Cu, 4.4; I, 1.1 g; Se, 0.1.

protocol, and the crop, ovary, kidney, liver, spleen, heart, proventriculus, gizzard, pancreas, and the entire intestine were excised aseptically and weighed. Relative organ weights (g/100 g of BW) were calculated (Edrington et al., 1997) and presented as percentages. The pH of the crop was determined by the insertion of a sterile glass pH electrode<sup>2</sup> through an incision in the crop wall, ensuring that the electrode remained in contact with the crop mucosal surface as described by Ramirez et al. (1997).

## Bone Shear Strength and Bone Ash

The right or left tibia of 1 hen was randomly chosen from a group of hens was excised from the fresh carcass and defleshed. The tibias were individually sealed in 4oz (≈113.5-g) plastic bags³ to minimize moisture loss and stored at 4°C. The tibias were dried at 105°C for 24 h, cooled to room temperature in a desiccator, and weighed to the nearest 0.01 g. Tibia shear strengths (breaking force divided by bone weight expressed as kg/g) (Shafer et al., 2001) were measured using an instron<sup>4</sup> with 50-kg-load cell at 50-kg-load range and a crosshead speed of 50 mm/ min with tibia supported on a 3.35-cm span. Tibia ash weights were determined by ashing in tared ceramic crucibles for 24 h at 615°C (Shafer et al., 2001). Percentage tibia ash was calculated by dividing tibia ash weight by tibia dry weight and multiplying by 100 (Al-Batshan et al., 1994).

## Zn Concentration in Kidney and Liver

Approximately 0.2 g (frozen) of each liver and kidney sample was dried at 75°C for 3 d and subsequently predigested in nitric acid for 3 d. The predigested sample was further digested for 2 h at 100°C using a microwave oven<sup>5</sup> (Davis, 1998; Daugherty, 2002). Atomic absorption spec-

<sup>&</sup>lt;sup>2</sup>Model 05669-20, Cole Palmer, Niles, IL.

<sup>&</sup>lt;sup>3</sup>Whirl-Pak bag, Nasco, Fort Atkinson, WI.

 $<sup>^4\</sup>mathrm{Model}$  1011, Instron Universal Testing Machine, Instron Corp., Canton, MA.

<sup>&</sup>lt;sup>5</sup>Inovative Microwave, CEM Corporation, Matthews, NC.

trophotometry<sup>6</sup> was used to determine Zn concentrations of the liver and kidney samples.

## Egg Production and Quality Parameters

Egg production and quality were monitored and compared after molting. Egg weight was measured using a balance and recorded to the nearest 0.01 g. Egg circumference was measured using a tape measure and recorded to the nearest 0.1 cm. Egg length and width were measured using a caliper and recorded to the nearest centimeter. Albumen height was measured using a micrometer and recorded to the nearest 0.1 mm. Shell thickness was evaluated using NaCl solutions, the specific gravity of which ranged from 1.065 to 1.110% in increments of 0.005%. This method using concentrations of NaCl solutions was based on an approach described by Keshavarz and Quimby (2002) with minimal modifications. Shell strength (kg) was also measured using an instron<sup>4</sup> with 50-kg-load cell at a 10-kg-load range and a crosshead speed of 50 mm/ min. Yolk color was expressed as Hunter color, L\*, a\*, and b\* (Hunter and Harold, 1987) using a colorimeter.<sup>7</sup> Each yolk pool was poured into a clean  $60 \times 15$ -mm glass Petri dish. A glass lid was placed flush against the yolk surface to prevent air pockets. The tip of the Chroma Meter measuring head was placed flat against the surface of the Petri dish and yolk reflective color was determined from the average of 3 consecutive pulses from the optical chamber of the Chroma Meter (Herber-McNeil and Van Elswyk, 1998). Daily egg production was monitored for 8 wk before molting and 12 wk after molting.

## Statistical Analysis

Data were analyzed using a one-way ANOVA to analyze the differences among treatment groups (feed withdrawal, Zn acetate, Zn propionate, and full-fed non-molted control) using general linear models procedures. Differences among treatment groups, when significant, were compared using Duncan's multiple range test. Level of significance used in all results was P < 0.05.

#### **RESULTS AND DISCUSSION**

#### Feed Intake

Hens fed the nonmolted control diet had (P < 0.05) greater feed intake (76.29 g/bird per day) than did hens fed 1% Zn in the form of Zn acetate or Zn propionate. However, there was no (P > 0.05) difference in feed intake between hens fed Zn acetate (22.61 g/bird per day) and hens fed Zn propionate (26.59 g/bird per day) (Table 2). There were approximately 65 and 70% reductions of feed intake in Zn propionate-fed hens and Zn acetate fed-hens,

<sup>6</sup>4000 Atomic Absorption, Perkin-Elmer, Norwalk, CT.

<sup>8</sup>SAS Institute Inc., Cary, NC, 1985.

respectively, when compared with the feed intake of hens fed nonmolted control layer ration.

Similar feed intake reduction was reported by Shippee and coworkers (1979) who observed that 1% Zn as Zn oxide or Zn acetate resulted in the average daily feed intake of 22 g/bird and 16 g/bird, respectively, during the second week of the forced resting period when hens reached 0% egg production. The reduced feed intake could be due to appetite depression (Brink et al., 1950) or low palatibility of high levels of Zn (Fox, 1989). It has also been reported that the reduced feed intake could be due to the ability of Zn cation (Zn<sup>2+</sup>) to induce follicular atresia and halt egg laying (Scott and Creger, 1976; Shippee et al., 1979; Berry and Brake, 1985; McCormick and Cunningham, 1987; Johnson and Brake 1992). Therefore, it is likely that the efficiency of high dietary Zn treatments as a method to induce molt in this study was directly related to the suppression of feed intake.

#### **BW Loss**

Hens undergoing feed withdrawal (25.12%) had more (P < 0.05) BW loss than hens fed 1% Zn in the form of Zn acetate or Zn propionate. However, there was no (P > 0.05) difference in BW loss between hens fed Zn acetate (15.52%) and hens fed Zn propionate (15.60%). Nonmolted hens had the least BW loss (1.15%) compared with other treatments of molted hens (Table 2).

The extent of BW loss by Zn acetate or Zn propionate feeding was similar to values in a previous study by McCormick and Cunningham (1987) who reported that BW losses over 4 d for fasted and zinc-fed hens were 16.4 and 15.2%, respectively. BW loss is a major factor contributing to the induced molting because BW loss affects on the successful results of an induced molting procedure (Brake and Thaxton, 1979; Brake et al., 1981; Brake and McDaniel, 1981; Baker et al., 1983). Baker and coworkers (1983) suggest that the extreme BW loss during induced molt is directly related to a hen's postmolt performance. Approximately 25% of BW loss by hens while subjected to feed withdrawal has been directly associated with decreased muscle weight, decreased liver weight, decreased use of adipose tissue, involution of the reproductive tissue, and greater reproductive regression (Brake and Thaxton, 1979; Berry and Brake, 1985).

## Ovarian Weight

Regression of the ovary is the most important factor for induced molt because the loss of reproductive weight may be linked to the overall rejuvenation process (Brake and Thaxton, 1979). Therefore, ovarian weight was measured in the current study as an indication of molting.

Hens fed 1% Zn in the form of Zn acetate (7.43 g) or Zn propionate (6.34 g) did not have (P > 0.05) different ovarian weights when compared with hens that were undergoing feed withdrawal (6.13 g). Nonmolted control hens had (P < 0.05) higher ovarian weights (31.04 g) when

<sup>&</sup>lt;sup>7</sup>Chroma Meter Model CR-200, Minolta Corp., Ramsey, NJ.

TABLE 2. Effect of molting and nonmolting diets on the crop pH, feed intake, body weight loss, and ovary weight of Single Comb White Leghorn hens

| Dietary treatment  | Crop pH   | Feed intake<br>(g/d)  | Body weight<br>lost (%)  | Ovary weight (g)   |
|--|---|---|--|--|
| Feed withdrawal <sup>1</sup><br>Zn acetate <sup>2</sup><br>Zn propionate <sup>3</sup><br>Layer ration <sup>4</sup> | $\begin{array}{l} 5.67  \pm  0.08^a \\ 5.98  \pm  0.09^a \\ 5.68  \pm  0.15^a \\ 4.89  \pm  0.08^b \end{array}$ | $\begin{array}{c} NA^5 \\ 22.61 \pm 3.27^b \\ 26.59 \pm 4.34^b \\ 76.29 \pm 6.36^a \end{array}$ | $\begin{array}{l} 25.12 \; \pm \; 1.51^{c} \\ 15.52 \; \pm \; 1.44^{b} \\ 15.66 \; \pm \; 1.43^{b} \\ 1.15 \; \pm \; 0.81^{a} \end{array}$ | $\begin{array}{c} 6.13  \pm  0.73^{\rm b} \\ 7.43  \pm  0.65^{\rm b} \\ 6.34  \pm  0.92^{\rm b} \\ 31.04  \pm  5.01^{\rm a} \end{array}$ |

a-cMeans within a column with no common superscripts differ significantly (P < 0.05).

compared with ovary weights from molted hens on dietary treatment (Table 2).

Berry and Brake (1985) reported that the ovarian weights of hens fed 2% Zn as Zn oxide for 8 d were 9.00 g. McCormick and Cunningham (1984) also reported that feeding of 1% Zn or 2% Zn as Zn oxide for 4 d resulted in reproductive involution (80% reduction in ovarian weights) of hens. Although we did not monitor or measure for reproductive involution in the current study, hens fed high dietary Zn as Zn acetate or Zn propionate may have experienced reproductive involution as indicated by substantial ovarian regression and reduced ovarian weight. The results may indicate that the major effects of high dietary Zn on the reproductive organs are somewhat linked to the general physiological state of hens fed high concentrations of Zn (Berry and Brake, 1985).

## Organ Weight

The proventriculus, gizzard, and intestines of chickens fed high doses of Zn appear normal and there are generally no histological changes (Dewar et al., 1983). According to Fosmire (1990), Zn is relatively nontoxic to animals and humans; both exhibit considerable tolerance to high intakes of Zn. However, 1 to 2% Zn in the diet results in anemia in rats (Duncan et al., 1953), reduced growth rates in chicks (Roberson and Schaible, 1960), and high mortality in chicks (Blalock and Hill, 1988). Therefore, the relative organ weights were measured in the

current study to investigate whether 1% Zn in feed may influence overall health of hens based on each organ when compared with those of nonmolted hens or hens molted by feed withdrawal.

There were no (P > 0.05) differences in relative weights of the heart, pancreas, intestine, or kidney among all molted hens and nonmolted hens (Table 3). Hens fed Zn propionate had (P > 0.05) higher relative liver weights (2.11%) than hens on feed withdrawal (1.74%). Brake and Thaxton (1979) and Berry and Brake (1985) noted that involution of the reproductive organs and the decrease in liver weight results in approximately 25% of the total BW reduction. Berry and Brake (1985) noted that the decrease in liver weight results from the removal of hepatic energy stores as glycogen and lipids that are metabolized in the liver. The liver is the target organ of yolk phospholipoprotein synthesis, which is dependent on ovarian steroids, primarily estrogen (Sturkie, 1976). McCormick and Cunningham (1984) noted that glycogen in the liver may not have been depleted because Zn interferes with insulin secretion. They also noted that the possible mechanism of Zn detoxification in the liver, including a high rate of bile production and synthesis of large quantities of the Zn storage protein metallothionein, may have contributed to the maintenance of liver weight.

Hens fed Zn propionate (0.13%) also exhibited (P > 0.05) higher relative spleen weights than hens undergoing feed withdrawal (0.10 g) or nonmolted control hens (0.10%). Hens fed Zn acetate (0.49%) had (P < 0.05) higher

TABLE 3. Effect of molting and nonmolting diets on the relative organ weight of Single Comb White Leghorn hens

| Dietary   | Liver   | Spleen   | Heart  | Proventriculus (%)  | Gizzard  | Pancreas  | Intestine  | Kidney   |
|---|---|--|--|---|--|---|--|--|
| treatment   | (%)   | (%)  | (%)  |   | (%)  | (%)   | (%)  | (%)  |
| Feed withdrawal <sup>2</sup> Zn acetate <sup>3</sup> Zn propionate <sup>4</sup> Layer ration <sup>5</sup> | $\begin{array}{c} 1.74 \ \pm \ 0.09^b \\ 1.90 \ \pm \ 0.10^{ab} \\ 2.11 \ \pm \ 0.09^a \\ 1.97 \ \pm \ 0.07^{ab} \end{array}$ | $\begin{array}{l} 0.10 \; \pm \; 0.006^{\rm b} \\ 0.12 \; \pm \; 0.009^{\rm ab} \\ 0.13 \; \pm \; 0.007^{\rm a} \\ 0.10 \; \pm \; 0.005^{\rm b} \end{array}$ | $\begin{array}{c} 0.47 \pm 0.03 \\ 0.411 \pm 0.018 \\ 0.409 \pm 0.02 \\ 0.39 \pm 0.03 \end{array}$ | $\begin{array}{c} 0.42\ \pm\ 0.01^b \\ 0.49\ \pm\ 0.02^a \\ 0.44\ \pm\ 0.02^b \\ 0.42\ \pm\ 0.01^b \end{array}$ | $\begin{array}{l} 1.76 \; \pm \; 0.01^{ab} \\ 1.66 \; \pm \; 0.08^{b} \\ 1.84 \; \pm \; 0.05^{a} \\ 1.42 \; \pm \; 0.03^{c} \end{array}$ | $\begin{array}{c} 0.16 \pm 0.02 \\ 0.19 \pm 0.02 \\ 0.17 \pm 0.01 \\ 0.19 \pm 0.02 \end{array}$ | 3.28 ± 0.26<br>3.70 ± 0.15<br>3.92 ± 0.20<br>3.35 ± 0.29 | 0.21 ± 0.02<br>0.28 ± 0.04<br>0.29 ± 0.03<br>0.27 ± 0.02 |

 $<sup>^{\</sup>mathrm{a-c}}$ Means within a column with no common superscripts differ significantly (P < 0.05).

<sup>&</sup>lt;sup>1</sup>Feed withdrawal = hens that were undergoing feed withdrawal for 9 d.

<sup>&</sup>lt;sup>2</sup>Zn acetate = hens that received a diet containing 10,000 mg/kg Zn as Zn acetate for 9 d.

 $<sup>^3</sup>$ Zn propionate = hens that received a diet containing 10,000 mg/kg Zn as Zn propionate for 9 d.

<sup>&</sup>lt;sup>4</sup>Layer ration = (nonmolted control) hens that received Texas A&M University (TAMU) layer ration for 9 d.

 $<sup>{}^{5}</sup>NA = not applicable.$ 

<sup>&</sup>lt;sup>1</sup>Relative organ weight (%) = (organ weight/100 g of body weight)  $\times$  100.

<sup>&</sup>lt;sup>2</sup>Feed withdrawal = hens that were undergoing feed withdrawal for 9 d.

<sup>&</sup>lt;sup>3</sup>Zn acetate = hens that received a diet containing 10,000 mg/kg Zn as Zn acetate for 9 d.

<sup>&</sup>lt;sup>4</sup>Zn propionate = hens that received a diet containing 10,000 mg/kg Zn as Zn propionate for 9 d.

<sup>&</sup>lt;sup>5</sup>Layer ration = (nonmolted control) hens that received Texas A&M University (TAMU) layer ration for 9 d.

TABLE 4. Effect of molting and nonmolting diets on the bone breaking strength and bone ash of Single Comb White Leghorn hens

| Dietary treatment   | Breaking strength (kg/g) <sup>1</sup>  | Bone ash (%) <sup>2</sup>   |
|---|--|---|
| Feed withdrawal <sup>3</sup> Zn acetate <sup>4</sup> Zn propionate <sup>5</sup> | $\begin{array}{c} 1.56 \pm 0.13 \\ 2.34 \pm 0.26 \\ 1.82 \pm 0.19 \end{array}$ | $\begin{array}{c} 39.98 \pm 1.62^{\rm b} \\ 46.78 \pm 1.79^{\rm a} \\ 46.01 \pm 1.08^{\rm a} \end{array}$ |
| Layer ration <sup>6</sup>   | $2.17 \pm 0.24$  | $45.32 \pm 0.94^{a}$  |

<sup>&</sup>lt;sup>a-c</sup>Means within a column with no common superscripts differ significantly (P < 0.05).

relative proventriculus weights than hens fed Zn propionate (0.44%), hens undergoing feed withdrawal (0.42%), or nonmolted hens (0.42%). However, hens fed Zn propionate (1.84%) had (P<0.05) higher relative gizzard weights than those fed Zn acetate (1.66%) or nonmolted hens (1.42%). Brake and Thaxton (1979) found that no consistent trends over trials were observed in the relative weights of the spleen from birds going through a forced molt.

No consistent trends were exhibited in the relative weights of organs in the current study. Compared with the hens on feed withdrawal or on the nonmolted control diet-, Zn-fed hens obtained statistically higher, lower, or the same value of relative organ weights, and which were dependent on organ type. Therefore, we could not definitively conclude that Zn feeding caused a harmful or toxic effect on these organs or on overall health condition of the hens.

#### Crop pH

Hens fed 1% Zn in the form of Zn acetate (5.98) or Zn propionate (5.68) chemical-added layer ration did not exhibit (P > 0.05) different crop pH levels when compared with hens that had undergone feed withdrawal (5.67). Nonmolted control hens (4.89) had a (P < 0.05) lower crop pH than molted hens (Table 2).

Humphrey and coworkers (1993) reported that when chickens are undergoing malnutrition or starvation, the pH of crop can increase due to decreased Lactobacillus fermentation within the crop. Feed withdrawal for 9 d resulted in a decrease in lactic acid in the crop, accompanied by an increase in crop pH (Durant et al., 1999). During the present study, the effects of dietary molting treatments on Lactobacillus populations and lactic acid concentrations in the crop were not determined. However, the results of the present study indicate that the increases in crop pH by dietary molting treatment for feed withdrawal, Zn acetate, or Zn propionate regimens may reduce the normal resident Lactobacillus population or lactic acid concentration in the crop. Zn acetate or Zn propionate feeding may be inhibitory to the *Lactobacillus* population due to the effect of Zn on microorganism growth. Dietary Zn may influence growth and infectivity of bacterial pathogens in animal. Park and coworkers (2002) recently reported that Zn compounds would inhibit in vitro aerobic or anaerobic growth of Salmonella typhimurium. Kubena et al. (2001) and Ricke et al. (2001) observed that molting diets containing high zinc decreased *S. enteritidis* colonization in laying hens when compared with hens undergoing feed withdrawal. Reduced feed intake may be the main factor causing the decrease in the *Lactobacillus* population (Humphrey et al., 1993; Corrier et al., 1999; Durant et al., 1999), thereby allowing the pH to rise in hens that are deprived of feed.

## Bone Breaking Strength and Bone Ash

Although there were no (P > 0.05) differences in tibia breaking strength among all molted hens and nonmolted

TABLE 5. Effect of molting and nonmolting diets on the concentrations of Zn in kidney and liver of Single Comb White Leghorn hens

| Dietary treatment   | Kidney<br>(ppm)  | Liver<br>(ppm)  |
|---|--|---|
| Feed withdrawal <sup>1</sup> Zn acetate <sup>2</sup> Zn propionate <sup>3</sup> | $170.58 \pm 44.8^{\mathrm{b}} \\ 361.59 \pm 80.34^{\mathrm{a}} \\ 486.04 \pm 35.00^{\mathrm{a}}$ | $\begin{array}{l} 552.13 \pm 86.10^{\rm b} \\ 1,546.14 \pm 230.63^{\rm a} \\ 1,661.59 \pm 321.19^{\rm a} \end{array}$ |
| Layer ration <sup>4</sup>   | $77.26 \pm 12.96^{\text{b}}$   | $127.98 \pm 56.32^{b}$  |

<sup>&</sup>lt;sup>a-b</sup>Means within a column with no common superscripts differ significantly (P < 0.05).

<sup>&</sup>lt;sup>1</sup>Bone breaking strength (kg/g) = tibia breaking force/tibia weight.

<sup>&</sup>lt;sup>2</sup>Bone ash (%) = (tibia ash weight/tibia dry weight)  $\times$  100.

<sup>&</sup>lt;sup>3</sup>Feed withdrawal = hens that were undergoing feed withdrawal for 9 d.

<sup>&</sup>lt;sup>4</sup>Zn acetate = hens that received a diet containing 10,000 mg/kg Zn as Zn acetate for 9 d.

<sup>&</sup>lt;sup>5</sup>Zn propionate = hens that received a diet containing 10,000 mg/kg Zn as Zn propionate for 9 d.

<sup>&</sup>lt;sup>6</sup>Layer ration = (nonmolted control) hens that received Texas A&M University (TAMU) layer ration for 9 d.

<sup>&</sup>lt;sup>1</sup>Feed withdrawal = hens that were undergoing feed withdrawal for 9 d.

<sup>&</sup>lt;sup>2</sup>Zn acetate = hens that received a diet containing 10,000 mg/kg zinc as Zn acetate for 9 d.

<sup>&</sup>lt;sup>3</sup>Zn propionate = hens that received a diet containing 10,000 mg/kg Zn as Zn propionate for 9 d.

<sup>&</sup>lt;sup>4</sup>Layer ration = (nonmolted control) hens that received Texas A&M University (TAMU) layer ration for 9 d.

hens, tibia ash (%) of hens on feed withdrawal were (P < 0.05) less than those of the other molted hens and non-molted hens (Table 4). The tibia ash (%) of feed-withdrawal hens, Zn acetate-fed hens, Zn propionate-fed hens, and nonmolted hens were 39.98, 45.32, 46.01, and 45.32, respectively.

Bone-breaking force (Crenshaw et al., 1981; Merkley, 1981; Ruff and Hughes, 1985), bone ash (Garlich et al., 1982), and bone ash concentration (Garlich et al., 1982; Cheng and Coon, 1990) have been used as indicators of bone status in the mineral nutrition of poultry and swine. In the current study, feed-withdrawal hens had the lowest tibia ash (%) among treatment groups. This result may indicate that hens undergoing feed withdrawal have smaller amounts of mineral in their tibias, which may be due to a rapid depletion of Ca reserves in fasting laying hens (Berry and Brake, 1985).

Medullary bone is the storage site for the Ca necessary for eggshell calcification (Mueller et al., 1964). Dietary Ca depletion of laying hens can cause a decrease in the amount in bone and an increase in the osteoid (Bloom et al., 1958; Zambonin Zallone and Teti, 1981). The excess Zn consumed by the hens may have interacted with blood and cellular Ca. Zn may have interacted with Ca absorption or metabolism, probably through interference with metal-containing enzymes (Underwood, 1977).

## Zn Concentrations in Kidney and Liver

The accumulation of Zn in various tissues is dependent on tissue type and Zn feeding (McCormick and Cunningham, 1987). Therefore, Zn concentrations in the kidney and liver in the current study were determined to investigate whether Zn feeding in the form of Zn acetate or Zn propionate would affect or increase Zn concentration in these tissues.

Hens fed 1% Zn in the form of Zn acetate (361.59 ppm) or Zn propionate (486.04 ppm) added to the layer ration had (P > 0.05) higher Zn concentrations in kidney when compared with hens that were undergoing feed withdrawal (170.58 ppm) or nonmolted hens (77.26 ppm) (Table 5). Hens fed 1% Zn in the form of Zn acetate (1,546.14 ppm) or Zn propionate (1,661.59 ppm) diets had (P > 0.05)higher Zn concentrations in the liver when compared with feed-withdrawal hens (552.13 ppm) or nonmolted hens (127.98 ppm) (Table 5). There was a significant increase in Zn concentrations in renal tissue of hens fed high Zn (1%) with approximately 3.7- and 5.3-fold increases in the renal Zn concentrations from Zn acetate-fed hens and Zn propionate-fed hens, respectively, compared with that of feed-withdrawal hens. Relative to kidney, the accumulation of Zn in liver was considerably higher in all molted hens and nonmolted hens. There were approximately 11and 12-fold increases in the hepatic Zn concentrations from Zn acetate-fed hens and Zn propionate-fed hens, respectively, compared with those of feed-withdrawal hens.

According to McCormick and Cunningham (1984), there are 4-fold, 10-fold, and 27-fold increases in the con-

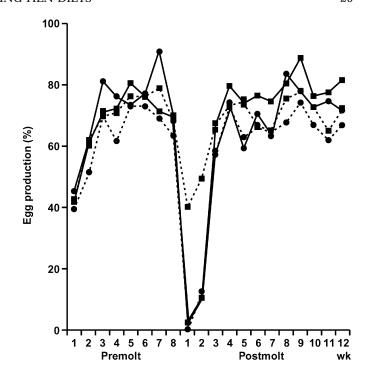
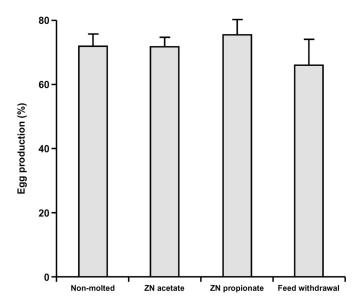


FIGURE 1. Percentage hen-day egg production from 4 treatments on a weekly basis during premolt and postmolt. Solid line with ■ represents Zn acetate (1% Zn) treatment, solid line with ● represents Zn propionate (1% Zn) treatment, dashed line with ■ represents nonmolted treatment, and dashed line with ● represents feed-withdrawal treatment.

centration of Zn in the kidney, liver, and pancreas, respectively, after feeding 1 or 2% Zn as Zn oxide for 4 d. McCormick and Cunningham (1984, 1987) also noted approximately 30% of the increase in Zn accumulation in kidney and liver was due to reduction of feed intake. Although urinary Zn excretion was not measured in the current study, we could speculate that urinary Zn concentrations may be increased during the molting process. The increase in urinary Zn excretion during starvation is considered to originate in skeletal muscle, which is being catabolized during starvation to provide a source of precursors for gluconeogenesis, amino acids for continuing protein synthetic activity by other tissues, and substrate for oxidation by the muscle (Fell et al., 1973). Elia et al. (1984) suggested that kidney, liver, and pancreas tissues have a buffering action with respect to the Zn released during the reduction of lean body mass, thus preventing excessive losses of Zn. Zinc concentrations in the liver and kidney may have accumulated due to the Zn (1%) feeding in the current study.

## **Egg Production**

Percentage hen-day egg production by 4 treatments on a weekly basis during premolt and postmolt is shown in Figure 1. As expected, nonmolted control hens had a (P < 0.05) higher level of egg production than hens from all other treatments in the first and second weeks after molting periods. However, there were no (P > 0.05) differences in overall egg production among molted hens and



**FIGURE 2.** Percentage average egg production after induced molting by 4 treatments (wk 3 to 12). Hens were grouped into fasted, fed 1% Zn as Zn acetate or Zn propionate, or fed the nonmolted control diet.

nonmolted control hens in relation to BW loss from wk 3 to the end of 12 wk after the molting periods (Figure 2). This finding may be because the age of the hens is an important factor in the overall response to induced molting (McCormick and Cunningham, 1987). Egg production in feed-withdrawal hens, Zn acetate-, and Zn propionate-fed hens, and nonmolted control hens from wk 3 to the end of 12 wk after molting was 66.38, 77.43, 70.31, and 71.04%, respectively. All molted hens reached more than 50% egg production from the third week to the end of the trial.

Zn propionate-fed hens had 0% egg production in the first week postmolt, whereas Zn acetate-fed hens had 2.04% egg production in the same week postmolt. From the second week postmolt, all molted groups of hens initiated egg production. The egg production by hens fed 1% Zn as Zn acetate or Zn propionate and hens undergoing feed withdrawal stopped by the fourth and fifth days, respectively (data not shown). Although hens fed 1% Zn in the form of Zn acetate or Zn propionate molted a

day earlier than hens undergoing feed withdrawal in the current study, overall egg production was not (P > 0.05)different for all molted hens. Shippee and coworkers (1979) reported that 1% Zn as Zn oxide or Zn acetate resulted in reduction of egg production from 60 to 0% within 6 d. Berry and Brake (1985) and Creger and Scott (1977) also reported that hens fed high Zn stopped ovulating a day earlier than fasted hens. The result may suggest that Zn has a direct effect on reproductive organs distinct from that of fasting and a combination effect of the suppression of feed intake resulting in regressive reproductive organs and direct suppression on the reproductive organs. Direct suppressive effect on the reproductive organs is independent of anorexia (Johnson and Brake, 1992). Overall egg production by hens fed 1% Zn in the form of Zn acetate or Zn propionate in the current study was higher than for hens undergoing feed withdrawal.

## Interior and Exterior Egg Quality

Interior and exterior egg quality in the current study were measured to investigate whether Zn feeding in the form of Zn acetate or Zn propionate would alter interior or exterior quality in eggs. There were no (P > 0.05) differences in Hunter L\* [lightness: 0 (black) to 100 (white)], a\* (+: redness, -: greenness), or b\* (+: yellowness, -: blue) values or albumen height (mm) among molted and nonmolted control hens during the postmolt periods (Table 6).

There were no (P>0.05) differences in egg width, length, or breaking strength for any of the treatments (Table 7). Hens undergoing feed withdrawal (64.92 g) had (P<0.05) lower egg weights than Zn propionate-fed hens (68.14 g) or nonmolted control diet-fed hens (68.37 g). Eggs from feed-withdrawal hens (14.28 cm) were also (P<0.05) smaller in circumference than those from nonmolted control hens (14.48 cm).

Thick shell eggs exhibit higher specific gravity values (Keshavarz and Quimby, 2002). In the current study, specific gravity was not different in any of treatments (data not shown), and overall specific gravity of shell eggs was 1.07%. This finding was similar to values from the study by Nesbeth and coworkers (1976) who reported that spe-

TABLE 6. Effect of molting and nonmolting diets on the internal egg quality of Single Comb White Leghorn hens during postmolt

| Dietary treatment   | Hunter L*1  | Hunter a*2  | Hunter b*3  | Albumen height<br>(mm)  |
|---|---|---|---|---|
| Feed withdrawal <sup>4</sup> Zn acetate <sup>5</sup> Zn propionate <sup>6</sup> Layer ration <sup>7</sup> | $\begin{array}{c} 56.69 \pm 0.45 \\ 56.20 \pm 0.58 \\ 56.75 \pm 0.41 \\ 55.67 \pm 0.42 \end{array}$ | $\begin{array}{c} -2.00  \pm  0.14 \\ -1.97  \pm  0.11 \\ -2.07  \pm  0.11 \\ -1.89  \pm  0.13 \end{array}$ | $\begin{array}{c} 42.55 \pm 0.48 \\ 41.35 \pm 0.74 \\ 41.90 \pm 0.51 \\ 42.51 \pm 0.67 \end{array}$ | $\begin{array}{c} 7.36 \ \pm \ 0.26 \\ 7.56 \ \pm \ 0.28 \\ 7.10 \ \pm \ 0.37 \\ 6.57 \ \pm \ 0.42 \end{array}$ |

<sup>&</sup>lt;sup>1</sup>Hunter color L\* values = lightness (0 = dark, 100 = bright).

<sup>&</sup>lt;sup>2</sup>Hunter color a\* values = redness/greenness (+ = red, - = green).

<sup>&</sup>lt;sup>3</sup>Hunter color b\* values = yellowness/blueness (+ = yellow, - = blue).

<sup>&</sup>lt;sup>4</sup>Feed withdrawal = hens that were undergoing feed withdrawal for 9 d.

<sup>&</sup>lt;sup>5</sup>Zn acetate = hens that received a diet containing 10,000 mg/kg Zn as Zn acetate for 9 d.

<sup>&</sup>lt;sup>6</sup>Zn propionate = hens that received a diet containing 10,000 mg/kg Zn as Zn propionate for 9 d.

<sup>&</sup>lt;sup>7</sup>Layer ration = (nonmolted control) hens that received Texas A&M University (TAMU) layer ration for 9 d.

TABLE 7. Effect of molting and nonmolting diets on the external egg quality of Single Comb White Leghorn hens during postmolt

| Diet treatment   | Weight<br>(g)  | Width<br>(cm)   | Length<br>(cm)  | Circumference<br>(cm)   | Breaking strength (kg/g)  |
|--|--|---|---|---|---|
| Feed withdrawal <sup>1</sup><br>Zn acetate <sup>2</sup><br>Zn propionate <sup>3</sup><br>Layer ration <sup>4</sup> | $\begin{array}{l} 64.92 \ \pm \ 0.85^{\rm b} \\ 66.35 \ \pm \ 0.78^{\rm ab} \\ 68.14 \ \pm \ 0.67^{\rm a} \\ 68.37 \ \pm \ 1.20^{\rm a} \end{array}$ | $\begin{array}{c} 4.79 \ \pm \ 0.11 \\ 4.78 \ \pm \ 0.10 \\ 4.96 \ \pm \ 0.13 \\ 4.88 \ \pm \ 0.14 \end{array}$ | $\begin{array}{c} 5.59 \pm 0.12 \\ 5.76 \pm 0.13 \\ 5.64 \pm 0.13 \\ 5.70 \pm 0.12 \end{array}$ | $\begin{array}{c} 14.28  \pm  0.06^{b} \\ 14.38  \pm  0.13^{ab} \\ 14.43  \pm  0.05^{ab} \\ 14.48  \pm  0.08^{a} \end{array}$ | $\begin{array}{l} 2.97  \pm  0.15^{\rm b} \\ 3.47  \pm  0.23^{\rm a} \\ 3.14  \pm  0.15^{\rm b} \\ 2.88  \pm  0.24^{\rm b} \end{array}$ |

<sup>&</sup>lt;sup>a,b</sup>Means within a column with no common superscripts differ significantly (P < 0.05).

cific gravity of eggs from a layer ration and a no-salt layer ration (molting diet) after molting was 1.073%. Shippee et al. (1979) reported that no significant differences in shell thickness were observed for Zn acetate and Zn oxide molting treatments during the postmolt periods. However, Bar and coworkers (2003) recently reported that shell thickness is significantly increased by Zn oxide (2.5% zinc) molting treatment.

The values of breaking strength by Zn acetate hens (3.47 kg/g) were (P > 0.05) higher than for Zn propionate hens (3.14 kg/g), feed-withdrawal hens (2.97 kg/g), or nonmolted hens (2.88 kg/g) (Table 7). This result may indicate that high-Zn diets with Zn acetate are effective in improving shell quality based on shell strength.

Most interior and exterior quality parameters were not different (P > 0.05) during 3 mo in postmolt for any of the treatments. However, egg weights of hens fed 1% Zn as Zn propionate (68.14 g) were higher (P < 0.05) than those of feed-withdrawal hens (64.92 g), but the egg weights of hens fed 1% Zn as Zn propionate were not (P > 0.05) different from those of hens fed 1% Zn as Zn acetate (66.35 g) or the nonmolted hens (68.37 g).

In general, egg size is larger during the second cycle than during the first cycle, and the shell quality and interior egg quality are better during the first cycle than during the second cycle (North and Bell, 1990). Zeelen (1975) also reported that egg size is increased significantly after induced molting with a higher percentage of eggs graded large. Zimmermann et al. (1987) reported that shell weight of eggs is improved only after molting. No significant differences were observed between feed and water restrictions and low-sodium diet in egg production, egg weight, interior egg quality, or shell quality during the postmolt periods (Naber et al., 1980; Said et al., 1984). However, Zn acetate and Zn oxide molting treatments resulted in improved egg Haugh units during the postmolt periods (Shippee et al., 1979).

The results in the current study indicate that egg qualities were not significantly (P>0.05) different in most parameters among all molted hens and full-fed layer ration hens. However, hens fed 1% Zn as Zn acetate had stronger shells, and hens fed 1% Zn as Zn propionate had higher egg weights than hens undergoing feed withdrawal. This higher egg weight may be an advantage

only if there is a consumer preference for the larger or heavier eggs in market (North and Bell, 1990).

## Summary

All laying hens treated with Zn propionate (1% Zn) in a layer diet had lower crop pH, completely stopped egg production by the fourth day in the molting process, and greatly reduced feed intake, BW loss, and ovary regression. Zinc-fed hens yielded statistically higher, lower, or similar values of relative organ weights that were dependent on organ type and yielded more tibia ash (%). Zinc concentrations in the liver and kidney accumulated, possibly due to Zn (1%) feeding. The overall egg qualities except for egg weight and egg production from Zn propionate were similar to other molting methods. Zinc accumulated in the liver and kidney. Therefore, dietary Zn propionate (1% zinc) as an alternative molting dietary Zn supplement was comparable in its effectiveness to induce molt. Future experiments are needed to examine the effect of Zn propionate on Salmonella infection in molted laying hens because induced molting without feed may increase the incidence of S. enteritidis in these hens (Holt and Porter, 1992; Holt, 1993; Holt et al., 1995).

#### **ACKNOWLEDGMENTS**

This research was supported by Kemin Americas, Inc., Des Moines, IA; TAES project G-8815; a Hatch grant H8311 administered by the Texas Agricultural Experiment Station; and USDA-NRI grant number 2001-02614. S. Y. Park was supported by a Pilgrim's Pride (Pittsburg, TX) endowed graduate fellowship.

#### REFERENCES

Al-Batshan, H. A., S. E. Scheideler, B. L. Black, J. D. Garlich, and K. E. Anderson. 1994. Duodenal calcium uptake, femur ash and eggshell quality decline with age and increase following molt. Poult. Sci. 73:1590–1596.

Baker, M., J. Brake, and G. R. McDaniel. 1983. The relationship between body weight loss during an induced molt and postmolt egg production, egg weight, and shell quality in cage layers. Poult. Sci. 62:409–413.

<sup>&</sup>lt;sup>1</sup>Relative organ weight = organ weight/100 g of body weight.

<sup>&</sup>lt;sup>2</sup>Feed withdrawal = hens that were undergoing feed withdrawal for 9 d.

<sup>&</sup>lt;sup>3</sup>Zn acetate = hens that received a diet containing 10,000 mg/kg Zn as Zn acetate for 9 d.

<sup>&</sup>lt;sup>4</sup>Zn propionate = hens that received a diet containing 10,000 mg/kg Zn as Zn propionate for 9 d.

<sup>&</sup>lt;sup>5</sup>Layer ration = (nonmolted control) hens that received Texas A&M University (TAMU) layer ration for 9 d.

Bar, A., V. Razaphkovsky, D. Shinder, and E. Vax. 2003. Alternative procedures for molt induction: Practical aspects. Poult. Sci. 82:543–550.

- Berry, W. D., and J. Brake. 1985. Comparison of parameters associated with molt induced by fasting, zinc, and low dietary sodium in caged layers. Poult. Sci. 64:2027–2036.
- Blalock, T. L., and C. H. Hill. 1988. Studies on the role of iron in zinc toxicity in chicks. Biol. Trace Element Res. 17:17–29.
- Bloom, M. A., L. V. Domm, A. V. Nalbandov, and W. Bloom. 1958. Medullary bone of laying chickens. Am. J. Anat. 102:411–451.
- Brake, J. 1993. Recent advances in induced molting. Poult. Sci. 72:929–931.
- Brake, J., M. Baker, and J. G. Mannix. 1981. Weight loss characteristics of the body, liver, ovary, oviduct, and uterine lipid during a forced molt and their relationship to performance. Poult. Sci. 60:1628 (Abstr.)
- Brake, J., and G. R. McDaniel. 1981. Factors affecting broiler breeder performance. 3. Relationship of body weight during fasting to postmolt performance. Poult. Sci. 60:726–729.
- Brake, J., and P. Thaxton. 1979. Physiological changes in caged layers during a forced molt. 2. Gross changes in organs. Poult. Sci. 58:707–716.
- Brink, M. F., D. E. Becker, S. W. Terrill, and A. H. Jensen. 1950. Zinc toxicity in the weanling pig. J. Anim. Sci. 18:836–842.
- Cave, N. A. G. 1982. Effect of dietary short- and medium-chain fatty acids on feed intake by chicks. Poult. Sci. 61:1147–1153.
- Cave, N. A. G. 1984. Effect of dietary propionic and lactic acids on feed intake by chicks. Poult. Sci. 63:131–134.Cheng, T. K., and C. N. Coon. 1990. Effect of calcium source,
- Cheng, T. K., and C. N. Coon. 1990. Effect of calcium source, particle size, limestone solubility *in vitro*, and calcium intake level on layer bone status and performance. Poult. Sci. 69:2214–2219.
- Corrier, D. E., J. A. Byrd, B. M. Hargis, M. E. Hume, R. H. Bailey, and L. H. Stanker. 1999. Survival of *Salmonella* in the crop contents of market-age broilers during feed withdrawal. Avian Dis. 43:453–460.
- Creger, C. R., and J. T. Scott. 1977. Dietary zinc as an effective resting agent for the laying hen. Poult. Sci. 56:1706. (Abstr.)
- Crenshaw, T. D., E. R. Peo, Jr., A. J. Lewis, and B. D. Moser. 1981. Bone strength as a trait of assessing mineralization in swine: A critical review of techniques involved. J. Anim. Sci. 53:827–835.
- Daugherty, S. B. 2002. Effects of prenatal energy supplementation and prenatal prebreeding trace mineral/vitamin E supplemental on calf health and reproductive performance in beef cows. M. S. Thesis. Texas A&M University, College Station, TX.
- Davis, M. S. 1998. Effects of supplemental copper on cellular and humoral immune response and performance of newly weaned calves. M. S. Thesis. Texas A&M University, College Station. TX.
- Dewar, W. A., P. A. L. Wight, R. A. Pearson, and M. J. Gentle. 1983. Toxic effects of high concentrations of zinc oxide in the diet of the chick and laying hen. Br. Poult. Sci. 24:397–404.
- Duncan, G. D., L. F. Gray, and L. J. Daniel. 1953. Effect of zinc on cytochrome oxidase activity. Proc. Soc. Exp. Biol. Med. 83:625–627.
- Durant, J. A., D. E. Corrier, J. A. Byrd, L. H. Stanker, and S. C. Ricke. 1999. Feed deprivation affects crop environment and modulates *Salmonella enteritidis* colonization and invasion of leghorn hens. Appl. Environ. Microbiol. 65:1919–1923.
- Edrington, T. S., L. F. Kubena, R. B. Harvey, and G. E. Rottinghaus. 1997. Influence of a superactivated charcoal on the toxic effects of aflatoxin or T-2 toxin in growing broilers. Poult. Sci. 76:1205–1211.
- Elia, M., C. Crozier, and G. Neale. 1984. Mineral metabolism during short-term starvation in man. Clin. Chim. Acta. 139:37–45.
- Fell, G. S., A. Fleck, D. P. Cuthbertson, K. Queen, C. Morrison, R. G. Bessent, and S. L. Husain. 1973. Urinary zinc levels as an indication of muscle catabolism. Lancet 1:280–282.

- Fosmire, G. J. 1990. Zinc toxicity. Am. J. Clin. Nutr. 51:225–227.Fox, M. R. S. 1989. Zinc excess. Pages 365–370 in Zinc in Human Biology. C. F. Mills, ed. Spring-Verlag, New York.
- Garlich, J., C. Morris, and J. Brake. 1982. External bone volume, ash, and fat-free dry weight of femurs of laying hens fed diets deficient or adequate in phosphorus. Poult. Sci. 61:1003–1006.
- Herber-McNeil, S. M., and M. E. Van Elswyk. 1998. Dietary marine algae maintains egg consumer acceptability while enhancing yolk color. Poult. Sci. 77:493–496.
- Holt, P. S.. 1993. Effect of induced molting on the susceptibility of White Leghorn hens to a Salmonella enteritidis infection. Avian Dis. 37:412–417.
- Holt, P. S., N. P. Macri, and R. E. Porter, Jr. 1995. Microbiogical analysis of the early *Salmonella enteritidis* infection in molted and unmolted laying hens. Avian Dis. 39:55–63.
- Holt, P. S., and R. E. Porter, Jr. 1992. Microbiological and histopathological effects of an induced-molt fasting procedure on a *Salmonella enteritidis* infection in chickens. Avian Dis. 36:610–618.
- Humphrey, T. J., A. Baskerville, A. Whitehead, B. Rowe, and A. Henley. 1993. Influence of feeding patterns on the artificial infection of laying hens with *Salmonella enteritidis* phage type 4. Vet. Rec. 132:407–409.
- Hunter, R. S., and R. W. Harold. 1987. The Measurement of Appearance. 2nd ed. Wiley Interscience, New York.
- Johnson, A. L., and J. Brake. 1992. Zinc-induced molt: Evidence for a direct inhibitory effect on granulosa cell steroidogenesis. Poult. Sci. 71:161–167.
- Keshavarz, K., and F. W. Quimby. 2002. An investigation of different molting techniques with an emphasis on animal welfare. J. Appl. Poult. Res. 11:54–67.
- Kubena, L. F., Y. M. Kwon, J. A. Byrd, C. L. Woodward, R. W. Moore, R. L. Ziprin, R. C. Anderson, D. J. Nisbet, and S. C. Ricke. 2001. Drinking water treatment and dietary treatment effects on *Salmonella enteritidis* in Leghorn hens during forced molt. Poult. Sci. 80(Suppl. 1):88. (Abstr.)
- McCormick, C. C., and D. L. Cunningham. 1984. Forced resting by high dietary zinc: Tissue zinc accumulation and reproductive organ weight changes. Poult. Sci. 63:1207–1212.
- McCormick, C. C., and D. L. Cunningham. 1987. Performance and physiological profiles of high dietary zinc and fasting as methods of inducing a forced rest: A direct comparison. Poult. Sci. 66:1007–1013.
- Merkley, J. W. 1981. A comparison of bone strengths from broilers reared under various conditions in coops and floor pens. Poult. Sci. 60:98–106.
- Mrosovsky, N., and D. F. Sherry. 1980. Animal anorexias. Science. 207:837–842.
- Mueller, W. J., R. Schraer, and H. Schraer. 1964. Calcium metabolism and skeletal dynamics of laying pullets. J. Nutr. 84:20–26.
- Naber, E. C., J. D. Latshaw, and G. A. Marsh. 1980. Use of low sodium diets for recycling of laying hens. Poult. Sci. 59:1643 (Abstr.)
- Nesbeth, W. G., C. R. Douglas, and R. H. Harms. 1976. The potential of dietary salt deficiency for the force resting of laying hens. Poult. Sci. 55:2375–2379.
- North, M. O., and D. D. Bell. 1990. Commercial Chicken Production Manual. 4th ed. Chapman Hall, New York.
- Paster, N. 1979. A commercial scale study of the efficiency of propionic acid and calcium propionate as fungistats in poultry feed. Poult. Sci. 58:572–576.
- Park, S. Y., C. L. Woodward, S. G. Birkhold, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2002. *In vitro* comparison of anaerobic acid and aerobic growth response of *Salmonella typhimurium* to zinc addition. J. Food Safety 22:219–229.
- Ramirez, G. A., L. L. Sarlin, D. J. Caldwell, C. R. Yezak, Jr., M. E. Hume, D. E. Corrier, J. R. DeLoach, and B. M. Hargis. 1997. Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. Poult. Sci. 76:654–656.

- Ricke, S. C., Y. M. Kwon, C. L. Woodward, J. A. Byrd, D. J. Nisbet, and L. F. Kubena. 2001. Limitation of *Salmonella enteritidis* colonization by diets containing low calcium and low zinc. Poult. Sci. 80(Suppl. 1):262. (Abstr.)
- Roberson, R. H., and P. J. Schaible. 1960. The tolerance of growing chicks for high levels of different forms of zinc. Poult. Sci. 39:893–896.
- Ryś, R., and J. Koreleski. 1974. The effect of dietary propionic acid on the requirement of chicks for vitamin  $B_{12}$ . Br. J. Nutr. 31:143-146.
- Ruff, C. R., and B. L. Hughes. 1985. Bone strength of heightrestricted broilers as affected by levels of calcium, phosphorus and manganese. Poult. Sci. 64:1628–1636.
- Said, N. W., T. W. Sullivan, H. R. Bird, and M. L. Sunde. 1984. A comparison of the effect of two forced molting methods on performance of two commercial strains of laying hens. Poult. Sci. 63:2399–2403.
- Scott, J. T., and C. R. Creger. 1976. The use of zinc as an effective molting agent in laying hens. Poult. Sci. 55:2089. (Abstr.)
- Shafer, D. J., R. P. Burgess, K. A. Conrad, J. F. Prochaska, and J. B. Carey. 2001. Characterization of alkaline hydroxide-

- preserved whole poultry as a dry byproduct meal. Poult. Sci. 80:1543-1548.
- Shippee, R. L., P. E. Stake, U. Koehn, J. L. Lambert, and R. W. Simmons, III. 1979. High dietary zinc or magnesium as forced-resting agents for laying hens. Poult. Sci. 58:949–954.
- Sturkie, P. D. 1976. Endocrine influences on lipid metabolism. Avian Physiology. 3rd ed. P. D. Sturkie, ed. Springer-Verlag, New York.
- Underwood, E. J. 1977. Zinc: Intermediary metabolism. Trace Elements in Human and Animal Nutrition. 4th ed. Academic Press, New York.
- Zambonin Zallone, A., and A. Teti. 1981. The osteoclasts of hen medullary bone under hypocalcaemic conditions. Anat. Embryol. 162:379–392.
- Zeelen, H. H. M., Jr. 1975. Technical and economic results from forced molting of laying hens. World's Poult. Sci. J. 31:57–67.
- Zimmermann, N. G., D. K. Andrews, and J. McGinnis. 1987. Comparisons of several induced molting methods on subsequent performance of Single Comb White Leghorn hens. Poult. Sci. 66:408–417.